

# Cytotoxic lymphocytes (CD8<sup>+</sup>) in the antrum mucosa in children with chronic *Helicobacter pylori* - related inflammation before and after bacteria eradication

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## Abstract

The authors assessed the expression of cytotoxic CD8<sup>+</sup> lymphocytes in the antrum mucosa of children with chronic *Helicobacter pylori* - related inflammation, before and after bacteria eradication. Biopsy specimens of gastric mucosa were evaluated in specimens, collected from 59 *H. pylori*-positive patients (Group I), 29 patients after *H. pylori* infection (Group II) and 18 *H. pylori*-negative children (Group III). The obtained specimens were assessed for infection and inflammation and the expression of CD8<sup>+</sup> lymphocytes was estimated, using monoclonal antibodies. The number of CD8<sup>+</sup> lymphocytes in the mucosa was counted.

The results of the study showed an increase in the expression of CD8<sup>+</sup> lymphocytes in children with *H. pylori* infection, in comparison to the values in children after bacteria eradication. The increased expression of CD8<sup>+</sup> lymphocytes correlated with the severity degree of antrum gastritis.

**Key words:** *Helicobacter pylori*, antrum mucosa, cytotoxic lymphocytes (CD8<sup>+</sup>).

## Introduction

CD8<sup>+</sup> phenotype cells compose most intraepithelial (IEL) lymphocytes of the digestive system, scattered among the epithelial cells. They are usually localized below the nuclei and their specific migration to the surface of the intestinal lumen may depend on the reaction of  $\alpha E\beta 7$  integrin (CD103) with E

cadherin of the epithelial cells. Approximately 15% of IEL have TCR  $\gamma/\delta$  receptors, very rarely present in the peripheral blood cells [1]. Some IEL present with features, characteristic for the natural cytotoxic cells but the role of these lymphocytes has not been explained completely, so far. It has been suggested that they may function as suppressors, preventing from a systemic immune response to food antigens. Moreover, they eliminate some pathogenic microorganisms and stimulate intestinal epithelium regeneration. CD8<sup>+</sup> lymphocytes of Th1 profile, producing IFN $\gamma$ , were described in the course of *H. pylori* infection [2, 3].

The aim of the study was to assess the expression of CD8<sup>+</sup> lymphocytes in the antrum mucosa in children with chronic inflammation of *H. pylori* aetiology, before and after bacteria eradication.

## Material and methods

Gastric mucosal biopsy specimens from the following three groups of individuals were studied by light microscopy: (I) 59 children (29 girls and 30 boys; the age range, 2-19 years) with chronic active inflammation of the gastric mucosa with *H. pylori* infection with positive IgG antibody anti- *H. pylori*; (II) 29 children (14 girls and 15 boys; the age range, 3-19 years) after *H. pylori* infection without either timely colonization or active inflammation of the gastric mucosa with maintaining positive IgG antibody anti- *H. pylori*. (III) 18 children (12 girls and 6 boys; the age range, 5-17 years) with gastrointestinal disease, without *H. pylori* infection, with correct level IgG antibody anti- *H. pylori*. In all the examined children, endoscopic examinations and histopathological studies of the stomach were performed. The sample sections were diagnosed, according to the Sydney System [4]. Urease test was performed in the course of endoscopy in all the children. Specimens were fixed in 10% buffered formalin. They were processed, oriented on edge, embedded in paraffin, cut in sequential 5- $\mu$ m sections,

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Photo 1. Antrum gastric mucosa. Immunohistochemical reaction with CD8<sup>+</sup>. Mag. 200x.

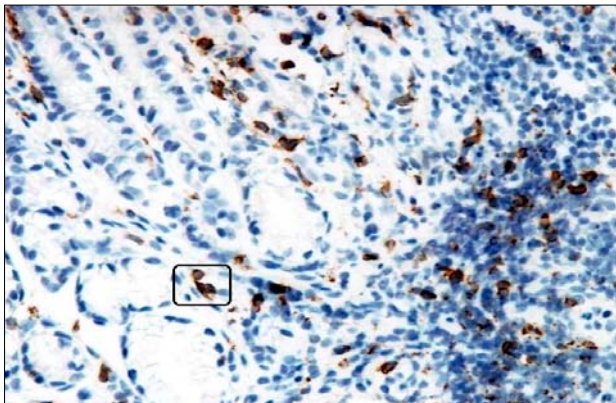


Figure 1. Correlation between the level of CD8<sup>+</sup> lymphocytes and the severity of antral gastritis in the children with Helicobacter pylori infection (group I)

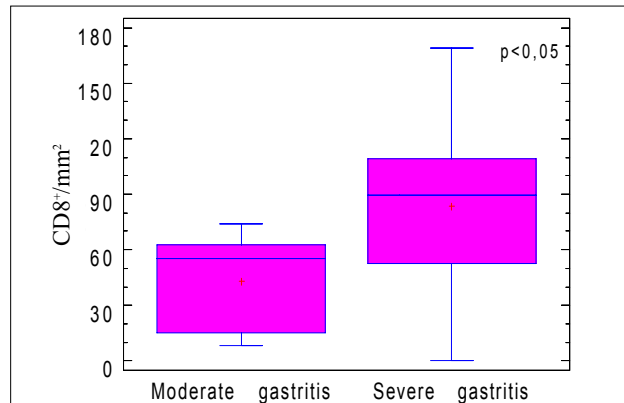


Table 1. CD8<sup>+</sup> lymphocytes in antrum mucosa of children.

Experimental groups	CD8 <sup>+</sup> lymphocytes in the antrum mucosa /mm <sup>2</sup>								
	Number of patients (N)	Result (min)	Result (max)	Arithmetic mean (x)	Median value (ME)	Moda	Standard deviation (SD)	Low quartile	High quartile
Group I	23	0	169	64.1	57	57	42.2	40.0	90.0
Group II	17	3	66	26.9	20	-	21.4	9.0	41.0
Group III	9	0	98	35.8	27	-	32.7	16.0	42.0

and stained by H+E for the evaluation of inflammation and Giemsa method was used to identify *H. pylori* bacteria (according to Gray) [5]. All the biopsy specimens from each of the study groups were stained by the immunohistochemical method for the evaluation of CD8<sup>+</sup> lymphocytes in the mucosa. The ABC method was used, according to commercial protocol. The number of CD8<sup>+</sup> lymphocytes in the mucosa was counted in discrete areas, measuring 0.785 mm<sup>2</sup>, each by using a light microscope. All the counts were performed, using a magnification of 200x. The numbers of positively stained cells were presented as mean values per 1 mm<sup>2</sup> of the analysed gastric section area. All the CD8<sup>+</sup> lymphocyte counts were performed by a single observer (I. K.), who was unaware of either the *H. pylori* status or the subject's clinical group.

An analysis of the preparations and their photographic documentation were performed with an Olympus Bx50 light microscope, with video circuit and a Pentium 120 PC computer with Lucia G (Nikon) software for microscope image analysis.

CD8<sup>+</sup> lymphocyte density was compared between the groups of studied subjects, using Student's 't' test. Statistical calculations included the arithmetic mean, standard deviation (SD), the median value (ME), the minimal result (min) and the maximal result (max). The levels of studied parameters were compared by means of Ch<sup>2</sup> test, either for independent or paired tests. All the differences were regarded significant at  $p < 0.05$ .

The obtained results have graphically been presented. Both the clinical examinations and all laboratory tests were performed in the children, following previous unrestrained consents of either their parents or legal protectors and after an approval of the Ethical Commission at the Medical University of Białystok.

## Results

According to Sydney's Classification, severe gastric mucosa inflammation was diagnosed in only 45.8% of the children in Group I, moderate - in 52.5% of the children with *H. pylori* infection and in 6.9% of the children after *H. pylori* infection eradication, mild - in 1.7% of the children in Group I, in 13.8% of the patients in Group II and in 15.4% of the children in the control group. Normal gastric mucosa of the antrum was found in 79.3% of the children from Group II and in 84.6% of the children in the control group.

A quantitative assessment of CD8<sup>+</sup> lymphocytes in the antrum mucosa (the mean number of cells per 1 mm<sup>2</sup> of specimen area), performed by immunohistochemical staining, revealed an increase in their expression in children with *H. pylori* infection (64.1 cells/mm<sup>2</sup>), in comparison with the results in children after eradication (26.9 cells/mm<sup>2</sup>) and those in the controls (35.8 cells/mm<sup>2</sup>) Table 1. and Photo 1.

Statistically significant differences were proved between the expressions of CD8<sup>+</sup> in the antrum mucosa in relation to the severity degrees of antrum gastritis ( $p < 0.05$ ) Fig. 1.

The expression of CD8<sup>+</sup> lymphocytes increases with intensification of the inflammatory process. In the *H. pylori* infected children with moderate antrum gastritis, the mean level of CD8<sup>+</sup> lymphocytes was 42.91 cells/mm<sup>2</sup>. In the case of severe antrum gastritis, the mean level was 83.58 cells/mm<sup>2</sup>. In the *H. pylori* infected children with moderate degree gastritis, the mean level of CD8<sup>+</sup> lymphocytes was 38.25 cells/mm<sup>2</sup>. In the case of severe degree inflammation, the mean expression of those lymphocytes was 69.58 cell/mm<sup>2</sup>.

## Discussion

The expression of CD8<sup>+</sup> lymphocytes in the antrum mucosa of children with gastritis in the course of *H. pylori* infection was the highest and differed significantly, in comparison with the expression in the group with past infection ( $p < 0.001$ ). It was proved that the expression of infiltrating CD8<sup>+</sup> cells increased with the severity of antrum gastritis.

Bamford et al. [3] reported an increase in both CD4<sup>+</sup> and CD8<sup>+</sup> expressions and an increased production of IFN- $\gamma$  and IL-12. They showed that Th<sub>1</sub> type response was predominant in T lymphocytes. Similarly, Sommer et al. [6] found CD4<sup>+</sup> cells of the Th<sub>1</sub> phenotype infiltrating the antrum and corpus mucosa in the course of *H. pylori*, which, according to the authors, may determine the incapability of the immune system of *H. pylori* eradication. Stromberg et al. [7] presented similar results, showing an increase in the levels of CD8<sup>+</sup> lymphocytes in the antrum mucosa in the course of *H. pylori*.

Terres et al. [8] demonstrated an increase in the expression of CD8<sup>+</sup> lymphocytes in the lamina propria in gastritis in the course of *H. pylori* infection. The expression of intraepithelial CD8<sup>+</sup> lymphocytes correlated with the severity of the inflammation, but it was independent of the degree of *H. pylori* colonization.

Summing up, it should be noted that an increased expres-

sion of CD8<sup>+</sup> cells in an inflammatory infiltrate in the antrum mucosa correlates with the severity of gastritis in children with *H. pylori* infection, in comparison respective values, observed in children after the bacteria eradication.

## References

1. Graham DY. Pathogenic mechanisms leading to *Helicobacter pylori*-induced inflammation. *Eur J Gastroenterol Hepatol*, 1992; 4: 9-16.
2. Lindholm C, Quiding-Jarbrink M, Lonroth H, Hamlet A, Svennerholm AM. Local cytokine response in *Helicobacter pylori* infected subjects. *Infect Immun*, 1998; 66: 5964-71.
3. Bamford KB, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, Brooks EG, Graham DY, Reyes VE, Ernst PB. Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. *Gastroenterology*, 1998; 114: 482-92.
4. Misiewicz JJ. The Sydney System: a new classification of gastritis. Introduction. *J Gastroenterol Hepatol*, 1991; 6: 207-8.
5. Gray SF, Wyatt JI, Rathbone BJ. Simplified techniques for identifying *Campylobacter pyloridis*. *J Clin Pathol*, 1986; 39: 1279.
6. Sommer F, Faller G, Konturek P, Kirchner T, Hahn EG, Zeus J, Rollinshoff M, Lohaff M. Antrum-and corpus mucosa- infiltrating CD4(+) lymphocytes in *Helicobacter pylori* gastritis display a Th1 phenotype. *Infect Immun*, 1998; 66: 5543-46.
7. Stromberg E, Lundgren A, Edebo A, Lundin S, Svennerholm AM, Lindholm C. Increased frequency of activated T-cells in the *Helicobacter pylori*-infected antrum and duodenum. *FEMS Immunol Med Microbiol*, 2003; 36: 159-68.
8. Terres A M, Pajares JM. An increased number of follicles containing activated CD69+ helper T cells and proliferating CD71+ B cells are found in *H. pylori*-infected gastric mucosa. *Am J Gastroenterol*, 1998; 93: 579-83.